

# NUCLEIC ACID METHODS FOR MICROBIAL DIAGNOSTICS AND PHYLOGENETICS MAY BE SUBJECTED TO DETRIMENTAL INHIBITORS AND MUTAGENS

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**Background:** Fungi, *inter alia*, are grown frequently on nutrients that support inhibitors and mutagens to produce nucleic acid (NA) for diagnostics and phylogenetics<sup>1,2,3</sup>. This situation is illogical as these may affect (a) the structure NA<sup>2</sup> and (b) PCR polymerases<sup>3</sup>. The situation is illustrated with the *idh* gene of patulin production.

**Objectives:** Rationalise NA analyse for diagnostics and phylogenetics.

## Methods:

**Data** PCR of nucleic acid using the model *idh* system for patulin in fungi.

**Interpretation** Using literature reviews to define problematic issues for NA analysis *per se*.

## Results:

1. Analysis of *idh* was successful for culture dependant PCR (CDP) and culture independent PCR (CIP).
2. A reversible inhibition of the PCR was observed in CDP presumably from inhibitors in cultures.
3. Inhibition was observed in CIP.
4. In some cases taxa which were predicted to be positive for *idh* were not, and *vice versa*.
5. A logical interpretation of this was that the gene was mutated by known mutagens/inhibitors in culture.
6. Brevianamides were detected in *Penicillium brevicompactum* cultures grown on the medium used to produce DNA.

## Conclusions:

1. *idh* PCR gave useful results in most cases.
2. It is illogical to grow microbes for NA analysis under conditions which support mutagens and inhibitors.
3. Such media need to be avoided otherwise reports on diagnostic and phylogenetic schemes can be undermined.
4. Work on *Aspergillus flavus* and related fungi are most vulnerable to this criticism, as they produce aflatoxins which are the most carcinogenic natural compounds.
5. Numerous fungi produce inhibitors and mutagens and so the problem is widespread.
6. There may be an equivalent situation for some bacteria.

## References:

1. Paterson, RRM. Mycological Research (2004), 108, 1431-1437.
2. Paterson, RRM, Lima, N. J Appl Microbiol(2009 In press)  
doi:10.1111/j.1365-2672.2008.04024.x
3. Paterson RRM. J Appl Microbiol (2007), 102, 1-10.